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RESEARCH NOTE

Distribution of the exfoliative toxin D gene in clinical *Staphylococcus aureus* isolates in France

O. Yamasaki[1], *A. Tristan*[1], *T. Yamaguchi*[2], *M. Sugai*[2], *G. Lina*[1], *M. Bes*[1], *F. Vandenesch*[1] *and J. Etienne*[1]

[1]Centre National de Référence des Staphylocoques, INSERM E0230, IFR62 Faculté de Médecine Laennec, Lyon, France and [2]Department of Bacteriology, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan

ABSTRACT

Exfoliative toxin D (ETD) was identified recently as a new exfoliative toxin serotype. Like other exfoliative toxins, ETD induces intra-epidermal cleavage through the granular layer of the epidermis of neonatal mice. The distribution of ETD production was investigated in *Staphylococcus aureus* isolates from infected and colonised patients in France. The *etd* gene was found in 55 (10.5%) of 522 isolates tested. Isolates responsible for bullous impetigo and generalised staphylococcal scalded skin syndrome did not harbour *etd*, but *etd* was significantly more frequent in isolates causing cutaneous abscesses and furuncles. Most *etd*- and Panton–Valentine leukocidin-positive strains belonged to the clone of community-acquired methicillin-resistant *S. aureus* spreading currently throughout France.

Corresponding author and reprint requests: J. Etienne, INSERM E0230, Faculté de Médecine Laennec, 7 rue Guillaume Paradin, 69372 Lyon cedex 08, France
E-mail: jetienne@univ-lyon1.fr

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Staphylococcus aureus produces a variety of extracellular toxins, including enterotoxins, toxic shock syndrome toxin (TSST1) and exfoliative toxins (ETs). ETs cause the epidermal cleavage seen in staphylococcal scalded skin syndrome (SSSS) and bullous impetigo [1]. Two major serological forms of ET, designated ETA and ETB, have been linked to human infections. A third ET (ETC) has been characterised and purified from a *S. aureus* isolate obtained from a horse [2]. ETs SHETA-B and ExhA-C are responsible for *Staphylococcus hyicus* exudative epidermitis in pigs [3]. An ET-like toxin has also been detected in *Staphylococcus intermedius* (SIET), and may have a pathogenetic role in canine pyoderma [4].

A new ET, designated ETD, is a 27-kDa protein with sequence similarities of 40% with ETA, 59% with ETB, 13% with ETC, 16% with SHET-A, and 63% with SHET-B [5]. Like other ETs, ETD causes epidermal blisters in newborn mice. The *etd* gene is located in tandem with *edin-B* (epidermal cell differentiation inhibitor B gene) in a new *S. aureus* pathogenicity island, designated the *etd* pathogenicity island [5]. The *etd* gene was detected in *S. aureus* isolates from 18 patients with suppurative diseases, but in only one of 88 isolates from patients with bullous impetigo or generalised SSSS [5,6]. The aim of the present study was to investigate the relationship between ETD and various human *S. aureus* diseases, including bullous impetigo and generalised SSSS.

The *S. aureus* isolates studied were referred to the French National Reference Centre for Staphylococci (Lyon, France) between 1 January 2003 and 31 December 2003 by hospitals throughout France. In total, 445 disease isolates and 77 carriage isolates (nose, eye, pharynx and vagina) were studied. A single isolate per patient was included when isolates were considered identical. Isolates were identified as *S. aureus* by their ability to coagulate citrated rabbit plasma (bioMérieux, Marcy-l'Etoile, France) and to produce clumping factor (Slidex Staph Kit; bioMérieux).

Genomic DNA was extracted following culture on agar plates or in brain–heart infusion broth by a standard phenol–chloroform extraction procedure. The *etd* gene was detected by PCR amplification with sense primer 5′-AACTATCATGTATCAAGG and antisense primer 5′-CAGAATTTCCCGACTCAG. The epidermal cell differentiation inhibitor *edin*-ABC genes were detected by PCR with the following primers mixed in an equimolar ratio: EDINAC-1, 5′-GATTTAGATGAGGCAACTAAATGGGG; EDINAC-2, 5′-CAGCGTATTCTGTGCCTCTAGG; EDINB-1, 5′-GACTTAGTTGAAGCTACTAAATGGGG; and EDINB-2, 5′-CAGCATATTCTGTCCCTCTAGG. Sequences specific for staphylococcal enterotoxin genes (*sea-e*, *seh*, *sek*, *sem*, *sel* and *seo*), the toxic shock syndrome toxin gene (*tst*), the exfoliative toxin A and B genes (*eta*, *etb*), the Panton–Valentine leukocidin (PVL) genes (*lukS*-PV–*lukF*-PV), the LukE–LukD leukotoxin gene (*lukE–lukD*), the γ-haemolysin gene (*hlg*), the γ-haemolysin variant gene (*hlgv*) and accessory gene regulator alleles (*agr*1–4) were all detected by PCR as described previously [7,8]. The *mecA* gene coding for methicillin resistance was detected by PCR as described by Murakami *et al*. [9]. All PCR products were resolved by electrophoresis through agarose 1.5% w/v gels, followed by staining with ethidium bromide and analysis. Pulsed-field gel electrophoresis (PFGE) was performed as described previously, and digitised patterns were compared using Taxotron (Institut Pasteur, Paris, France) software. A PFGE type corresponded to isolates with PFGE patterns with >80% similarity. The χ^2 test was used to compare proportions between variable categories, with all analyses performed with SPSS software v.11.5 (SPSS Inc. Chicago, IL, USA).

The *etd* gene was detected in 55 (10.5%) of the 522 *S. aureus* isolates. It was detected more frequently in the present study than were *eta* and *etb* in previous studies (5.1–6%) [10,11]. Forty-eight of the 445 disease isolates, and seven of the 77 colonisation isolates, were *etd*-positive (Table 1). None of the isolates responsible for bullous impetigo or generalised SSSS harboured *etd*, thereby confirming the original results of Yamaguchi *et al*. [5], who found that only one of 88 isolates from Japanese patients with bullous impetigo produced ETD.

Table 1. Presence of the *etd* gene in 522 *Staphylococcus aureus* isolates associated with various clinical syndromes

Colonisation/infection (no. of isolates tested)	No. (%) of *etd*-positive isolates	p value
Colonisation (77)	7 (9)	NS
Infections (445)	48 (10.5)	
Skin infections (188)	41 (74)	0.001
Bullous impetigo (32)	0 (0)	NS
Generalised SSSS (14)	0 (0)	NS
Cutaneous abscess (54)	18 (33)	0.06
Furuncle (21)	9 (43)	NS
Finger pulp infection (8)	4 (50)	NS
Secondary skin infection (59)	10 (17)	NS
Other infections (257)	7 (13)	0.003
Mastoiditis (8)	2 (25)	NS
Pneumonia (43)	1 (2)	NS
Sepsis (58)	3 (5)	NS
Osteoarthritis (23)	1 (4)	NS
Osteomyelitis (11)	0 (0)	NS
Endocarditis (19)	0 (0)	NS
Other (95)	0 (0)	NS

NS, not significant; SSSS, Staphylococcal scalded skin syndrome.

SSSS is associated classically with ETA or ETB. The *etd* gene was detected mainly in isolates (n = 41) from patients with skin and soft-tissue infections, such as furuncles, abscesses and finger pulp infections (p 0.001), which are caused rarely by *S. aureus* strains harbouring *eta* or *etb*, but are associated strongly with production of PVL [7], and was detected only rarely in isolates from patients with other suppurative diseases. It is unclear whether ETD contributes directly to the pathogenesis of these diseases, but the present findings support the hypothesis that ETD may disrupt the cutaneous epithelial barrier and thereby contribute to bacterial spread [12]. Indeed, purified ETD induces intra-epidermal cleavage through the granular layer of the epidermis of neonatal mice in a manner similar to ETA [13] and ETB [14]. As for other ETs, a possible substrate for ETD is desmoglein 1, a desmosome trans-membrane glycoprotein belonging to the cadherin gene superfamily [5,14].

The 55 *etd*-positive isolates were negative for eta and *etb*, and belonged to only two PFGE types (data not shown). Forty-nine isolates belonged to type I (48 with an *etd*$^+$, *lukS*-PV–*lukF*-PV$^+$, *mecA*$^+$ genotype, and one with the same genotype except that it was *mecA*$^-$). The 48 PVL-positive isolates belonged to a clone of community-acquired (CA) methicillin-resistant *S. aureus* (MRSA) of sequence type 80 spreading currently throughout Europe [15,16]. They harboured the *agr*3 allele, and were otherwise identical as regards their toxin gene profiles (negative for *tst*, *hlg and* all enterotoxin genes, and positive for *lukE–lukD* and *hlgv*). The second PFGE type (II) comprised six *agr*1 isolates (one *lukS*-PV–*lukF*-PV$^+$, *mec*A$^-$, *etd*$^+$ isolate and five *lukS*-PV–*lukF*-PV$^-$, *mecA*$^-$, *etd*$^+$ isolates) (Table 2).

All *etd*-positive isolates were also *edin*-positive. EDIN belongs to the ADP-ribosyltransferase-modifying Rho GTPases8. In-vitro, EDIN induces epidermal hyperplasia [17]. Recent studies suggest that the skin of patients with atopic dermatitis and epidermal hyperplasia has increased binding avidity for *S. aureus* [18]. It is therefore conceivable that EDIN facilitates *S. aureus* skin infection, possibly in cooperation with EDIN-B [5]. The fact that *etd* and *edin* have not been detected in clones of CA-MRSA spreading in the USA or Oceania suggests that *etd* and

Table 2. Accessory gene profiles of *Staphylococcus aureus* isolates positive for exfoliative toxin D gene

Genotypes	No. of isolates	*agr* allele	*edin*	*eta*, *etb*	*lukE–lukD*	Enterotoxin genes: *sea-e, seh, sek, sem, sel, seo*	*tst*	*hlg*	*hlgv*	PFGE
etd$^+$, *lukS*-PV–*lukF*-PV$^+$, *mecA*$^+$	48	3	+	–	+	–	–	–	+	Clone I
etd$^+$,	5	1	+	–	+	*s, sel, sem, seo*	–	–	+	Clone II
lukS-PV–*lukF*-PV$^-$,		1	+	–	+	*sem, seo*	–	–	+	Clone II
mecA$^-$		1	+	–	+	*seb, sem, seo*	–	–	+	Clone II
		1	+	–	+	*seb, sem, seo*	–	–	+	Clone II
		1	+	–	+	*seb, sem, seo*	–	–	+	Clone II
etd$^+$,	2	1	+	–	+	*seb, sem, seo*	–	–	+	Clone II
lukS-PV–*lukF*-PV$^+$, *mecA*$^-$		3	+	–	+	–	–	–	+	Clone I

agr, accessory gene regulator; *edin*, epidermal cell differentiation inhibitor genes; *eta*, *etb*, exfoliative toxin A and B genes; *lukE–lukD*, leukotoxin ED genes; *sea-e*, *seh*, *sek*, *sel*, *sem*, *seo*, genes for enterotoxins SEA–SEA, SEH, SEK, SEL, SEM and SEO, respectively; *tst*, toxic shock syndrome toxin gene; *hlg*, γ-haemolysin gene; *hlgv*, γ-haemolysin-variant gene; *etd*, exfoliative toxin D gene; *lukS*-PV–*lukF*-PV, Panton–Valentine leukocidin genes; *mecA*, methicillin resistance gene; +, present; –, absent; PFGE, pulsed-field gel electrophoresis.

edin may be specific genetic traits of European CA-MRSA.

In conclusion, *etd* was not associated currently with bullous impetigo or generalised SSSS, both of which are caused by ETs. However, *etd* was associated with *edin* and *lukS*-PV–*lukF*-PV in CA-MRSA strains spreading throughout Europe [15], and this combination may contribute to epidemicity and virulence.

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RESEARCH NOTE

Increased conjugation frequencies in clinical *Enterococcus faecium* strains harbouring the enterococcal surface protein gene *esp*

B. Lund[1,2], *H. Billström*[1] *and C. Edlund*[1,2]

[1]Division of Clinical Bacteriology, Department of Laboratory Medicine, Huddinge University Hospital, Karolinska Institutet and [2]Södertörns högskola University College, Stockholm, Sweden

ABSTRACT

This study compared the in-vitro ability of *Enterococcus faecium* isolates of different origin to acquire *vanA* by conjugation in relation to the occurrence of the *esp* gene. In total, 29 clinical isolates (15/29 *esp*+), 30 normal intestinal microflora isolates (2/30 *esp*+) and one probiotic strain

Corresponding author and reprint requests: C. Edlund, F82, Division of Clinical Bacteriology, Karolinska University Hospital, Huddinge, SE-141 86 Stockholm, Sweden
E-mail: charlotta.edlund@labmed.ki.se